Developmental Validation of the Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® FSC Instrument

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Maxwell® FSC Instrument (Cat.# AS4600).

Introduction

Short Tandem Repeat (STR) analysis is a useful DNA profiling technique for human DNA identification. The ability to compare profiles of DNA extracted from a crime scene with those of reference samples is dependent on the STR profile quality. Factors that can influence STR profile quality include presence of PCR inhibitors in the sample and introduction of exogenous DNA into the sample. A DNA extraction procedure that yields clean DNA is crucial because forensic samples have an increased propensity to contain environmental components that may negatively affect the profile quality. Automation of a DNA extraction procedure combined with an easy-to-follow protocol decreases hands-on time and reduces risk of sample contamination, thus also increasing the potential DNA profile quality.

To address the need for high-quality STR profiles, the Maxwell® FSC (Forensic Sample Concentrator) Instrument was developed for automated DNA extraction from forensic-type samples. The instrument is supplied with preprogrammed DNA purification procedures and uses prefilled reagent cartridges, resulting in an easy-to-follow extraction protocol. The Maxwell® FSC DNA IQ™ Casework Kit uses DNA IQ™ Resin to produce high-purity nucleic acid for downstream quantification and STR amplification. DNA IQ™ chemistry uses paramagnetic particles to efficiently prepare clean samples for STR analysis. The Maxwell® FSC DNA IQ™ Casework Kit can be used to extract DNA from a variety of sample types including blood, semen stains and "touch" DNA samples. The DNA IQ™ Resin is used extensively with manual methods and on large-scale automation platforms (1–5). The use of Maxwell® FSC DNA IQ™ chemistry with the Maxwell® FSC Instrument, as described in this paper, is well suited for medium-throughput DNA extraction from forensically relevant samples because the instrument can process up to 16 samples in approximately 30 minutes. Forensic-grade plastics and improved spin baskets are available and recommended for use with the Maxwell® FSC DNA IQ™ Casework Kit to further minimize the risk of contamination.

In this article, we report on the validation studies performed during development of the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit for genomic DNA extraction from forensically relevant biological samples. These studies are based on requirements listed in the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (6) and guidelines outlined by the Scientific Working Group on DNA Analysis Methods (7). Validation results reiterate the suitability of the Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® FSC Instrument for use with forensic casework samples to maximize STR profile quality.

Materials and Methods

Samples

Human blood, saliva and buccal swabs were obtained from volunteers. Semen samples were purchased from Lee Biosolutions, Inc (MO, USA). Casework-type samples were collected and processed by Johnson County Sheriff's Office Criminalistics Laboratory (KS, USA). Maxwell® FSC DNA IQ™ Casework Kit, Casework Extraction Kit, PowerQuant® and PowerPlex® Fusion 6C Systems were obtained from Promega Corporation (WI, USA). All other chemicals used in this study were of analytical grade.

Preprocessing

Sample preprocessing was performed using the Casework Extraction Kit, which contains Casework Extraction Buffer, Proteinase K and 1-Thioglycerol. The resulting lysate was purified using reagents and components in the Maxwell® FSC DNA IQ™ Casework Kit. Detailed preprocessing and purification protocols for solid and liquid samples are available in the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual* TM499 (8).

Casework samples containing semen were preprocessed to separate the sperm fraction from the non-sperm fraction. Non-sperm digest buffer was prepared by adding 390µl of Stain Extraction Buffer with Sarkosyl and 10µl of 18mg/ml Proteinase K for a final volume of 400µl per sample. Each sample was incubated at 56°C for two hours before transferring the substrate to a spin basket assembly and centrifuging. The non-sperm fraction was separated, and the sperm pellet was washed with Stain Extraction Buffer. The sperm fraction and non-sperm fraction were then processed according to the Differential Extraction Samples section of the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual* TM499 (8).

DNA Extraction from Solid Substrates

All swab samples were processed using CW Spin Baskets. A CW Spin Basket was placed in a CW Microfuge Tube, and the solid substrate (swab head) was placed at the bottom of the CW Spin Basket. The extraction mix was prepared by adding 286µl of Casework Extraction Buffer, 10µl of 18mg/ml Proteinase K and 4µl of 1-Thioglycerol for a final volume of 300µl per sample. Each sample was incubated at 56°C for 30 minutes and centrifuged at maximum speed for 2 minutes at room temperature. The CW Spin Baskets were then discarded, and 200µl of Lysis Buffer was added to each tube containing extract. Following thorough mixing, the samples were added to the Maxwell® FSC Cartridge, and the instrument protocol was initiated according to manufacturer instructions. All samples were eluted to a final volume of 50µl.

DNA Extraction from Liquid Samples

For the sensitivity study, 286µl of Casework Extraction Buffer, 10µl of Proteinase K (18mg/ml) and 4µl of 1-Thioglycerol were added to 100µl of liquid sample. For the reproducibility study, 376µl of Casework Extraction Buffer, 10µl of Proteinase K and 4µl of 1-Thioglycerol were added to 10µl of liquid sample. Each sample was mixed thoroughly and incubated at 56°C for 30 minutes. Following incubation, 200µl of Lysis Buffer was added to each sample, which was then added to the Maxwell® FSC Cartridge. The instrument protocol was initiated according to manufacturer instructions. All samples were eluted to a final volume of 50µl.

DNA Quantification

The extracted DNA from each sample was quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System following the protocol detailed in the *PowerQuant® System Technical Manual* TMD047 (9). The following sample quality flags were monitored throughout the studies: [Autosomal]/[Degradation] ratio, [Autosomal]/[Y] ratio and IPC (internal positive control) C_q. These quality flags provide information about the sample and probable STR profile quality. A threshold of 2 was used for the [Auto]/[D] and [Auto]/[Y] ratios to flag a sample as possibly degraded or as a potential mixture, respectively. A shift of 0.3 for the IPC C_q was used to flag a sample for possible inhibition.

STR Analysis

Samples were amplified with PowerPlex® Fusion 6C System following the protocol detailed in the *PowerPlex® Fusion 6C System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* TMD045 (10). A total of 1ng or up to 15µl of sample was added per amplification reaction. Samples were amplified on a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was used for electrophoresis. All samples except those in the casework study were injected at 1.2kV for 24 seconds on the 3500xL Genetic Analyzer. Casework samples were injected at 1.2kV for 15 seconds on the 3500 Genetic Analyzer. Data were analyzed with GeneMapper® *ID*-X Software v1.5 using 100RFU as the analysis threshold.

Sensitivity Study

To evaluate the linear range of DNA extracted, we performed a sensitivity study. Samples from two individuals containing 0.1µl, 0.5µl, 1.0µl, 5.0µl, 10µl, 50µl and 100µl of liquid blood were processed in triplicate as described in the Preprocessing and DNA Extraction from Liquid Samples sections. All samples were diluted to a final volume of 100µl with 1X PBS prior to extraction.

Reproducibility Study

We assessed the reproducibility of DNA quantity and quality obtained from replicate samples. DNA from 10µl of body fluid (blood, saliva and semen) was extracted in quadruplicate as described in the Preprocessing and DNA Extraction from Liquid Samples sections to compare replicates within an extraction run. The extraction setup was repeated to compare replicates between extraction runs.

Contamination Study

To assess sample-to-sample contamination, extraction blanks were processed along with liquid body fluid samples (blood, saliva and semen) in an alternating pattern on the Maxwell® FSC Instrument.

Mixture Study

A mixture study was performed to show that DNA from mixed donor cells in a variety of ratios can be effectively extracted and purified. Two mixture series were prepared by combining male and female blood (by volume) to obtain M:F ratios of 1:1, 1:5, 1:10, 1:20 and 1:50. Each sample contained 10µl of the blood mixture dried on a cotton swab. Mixture samples were extracted on the Maxwell® FSC Instrument as described in the Preprocessing and DNA Extraction from Solid Substrates sections.

Inhibition Study

We performed an inhibition study to show that DNA can be effectively purified in the presence of inhibitors commonly encountered in forensic casework. Blood (10µl) was added to swabs along with an appropriate volume of inhibitor solution (humic acid and hematin) and allowed to dry before extraction. The following volumes of inhibitors were tested: 3µl and 6µl of 5mg/ml stock humic acid; 60µl and 120µl of 2mM stock hematin.

Known and Casework Samples

Known buccal swabs and a variety of casework-type samples were included in this study to evaluate the performance in yielding pure and amplifiable DNA. Known samples consisted of duplicate buccal swabs from four female and three male individuals. Casework samples included mock evidence such as touch samples, differentially extracted samples, saliva and blood.

Results and Discussion

Sensitivity Study

Quantifiable levels of DNA were detected from all extracted blood samples in the sensitivity study. DNA yield extracted from body fluids may vary depending on sample type and individual. A total yield of 1.46ng and 1.11ng of DNA was extracted from 0.1µl of blood for Individual 1 and Individual 2, respectively (Table 1). The results show a linear relationship between the volume of blood and the concentration of DNA extracted for input volumes between 0.1–10µl (Figure 1). DNA IQ™ Resin uses a nonporous silica technology that has a finite DNA binding capacity. The limits of the system were tested by including larger volumes of blood such as 50µl and 100µl. The 100µl blood sample yielded less DNA than the 50µl blood sample, which may indicate the presence of a large quantity of sample components competing for binding to the resin (11). IPC C_q flags were not observed in any samples, indicating DNA IQ™ chemistry yields clean DNA even from large sample inputs (data not shown). All samples from this study yielded full DNA profiles when amplified with PowerPlex® Fusion 6C System (data not shown).

Table 1. Average total autosomal DNA yield ±1 standard deviation compared to volume of blood extracted.

	Average Total Yield (ng)	
Volume of Blood (µl)	Individual 1	Individual 2
100	291.03 ± 24.48	284.14 ± 86.51
50	349.54 ± 27.30	355.17 ± 77.26
10	127.19 ± 8.82	174.01 ± 41.88
5	88.21 ± 30.50	114.01 ± 15.91
1	16.78 ± 5.96	18.42 ± 4.14
0.5	11.06 ± 0.82	4.06 ± 0.70
0.1	1.46 ± 0.70	1.11 ± 0.24

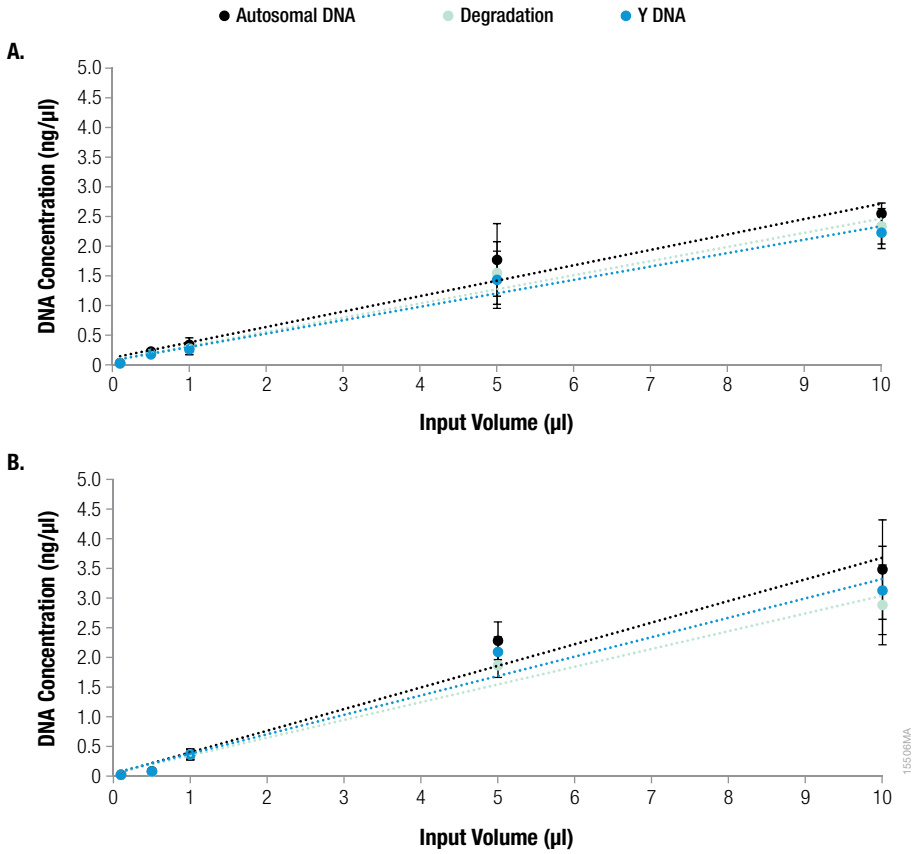

Figure 1. Linearity of DNA concentration from each extraction for Individual 1 (Panel A) and Individual 2 (Panel B). The X axis represents the volume of blood used for sample extraction. The Y axis represents the average DNA concentration (Autosomal, Degradation and Y DNA targets) from the PowerQuant® System. The error bars represent ±1 standard deviation. Samples were extracted using the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System.

Reproducibility Study

The DNA concentration variability within runs and between runs was similar for each of the body fluids tested (Table 2). No IPC C_q flag was detected in any of the samples, indicating the DNA obtained was pure. Amplification with the PowerPlex® Fusion 6C System showed complete STR profiles with consistent genotypes (data not shown).

Contamination Study

No DNA was detected from the extraction blanks run in the contamination study using the PowerQuant® System. Amplification of extraction blank samples with the PowerPlex® Fusion 6C System generated no amplification products (data not shown).

Mixture Study

The results show the ability of the system to extract DNA from both individuals in the mixture. A progressive decrease in the male component of the mixture correlated to a decrease in male DNA concentration and a concomitant increase in [Auto]/[Y] ratio (Figure 2).

Inhibition Study

No IPC C_q flags were detected in the eluted DNA from the inhibition study (Figure 3), indicating the DNA IQ™ purification process removed inhibitors. Full profiles were recovered, and expected mean peak heights were obtained from all samples when amplified with the PowerPlex® Fusion 6C System. Representative electropherogram data are shown in Figure 4.

Table 2. Average concentration, standard deviation and %CV across targets in PowerQuant® System for replicates within runs and between runs for body fluids.

	Average [Autosomal] (ng/µl)	[Autosomal] CV	Average [Degradation] (ng/µl)	[Degradation] CV	Average [Y] (ng/µl)	[Y] CV	Average IPC C_q	IPC C_q CV
Blood Extraction								
Run #1 (N=4)	2.86 ± 0.22	7.8%	2.47 ± 0.15	6.2%	2.49 ± 0.21	8.5%	20.44 ± 0.07	0.4%
Run #2 (N=4)	3.97 ± 1.04	26.1%	3.05 ± 0.88	28.8%	3.10 ± 0.93	29.9%	20.31 ± 0.07	0.3%
Inter-Run (N=8)	3.41 ± 0.94	27.4%	2.76 ± 0.69	25.1%	2.79 ± 0.74	26.4%	20.37 ± 0.09	0.5%
Saliva Extraction								
Run #1 (N=4)	0.0398 ± 0.0103	25.9%	0.0292 ± 0.0066	22.8%	0.0350 ± 0.0084	23.9%	20.39 ± 0.04	0.2%
Run #2 (N=4)	0.0485 ± 0.0172	35.5%	0.0377 ± 0.0132	34.9%	0.0479 ± 0.0181	37.8%	20.37 ± 0.05	0.2%
Inter-Run (N=8)	0.0441 ± 0.0148	33.6%	0.0334 ± 0.0113	33.7%	0.0415 ± 0.0155	37.4%	20.38 ± 0.05	0.2%
Semen Extraction								
Run #1 (N=4)	11.32 ± 3.68	32.5%	8.18 ± 2.70	33.0%	9.02 ± 2.84	31.5%	20.33 ± 0.01	0.1%
Run #2 (N=4)	9.36 ± 3.23	34.5%	8.59 ± 2.94	34.2%	8.44 ± 2.97	35.3%	20.55 ± 0.08	0.4%
Inter-Run (N=8)	10.35 ± 3.60	34.8%	8.39 ± 2.83	33.8%	8.73 ± 2.92	33.5%	20.45 ± 0.12	0.6%

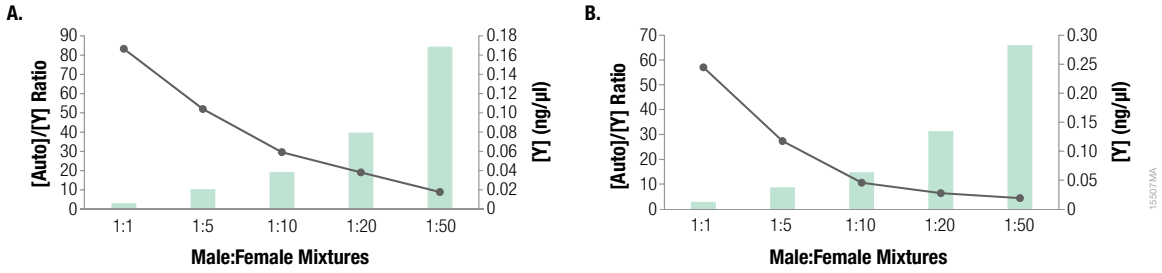

Figure 2. Quantification results for two male/female mixture sets. The X axis represents the ratio of male to female blood that was used in the sample. The left Y axis represents the average ratio of autosomal DNA to male DNA (indicated by the bars). The right Y axis represents the average male DNA concentration in ng/µl (indicated by the line). Samples were extracted using the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System.

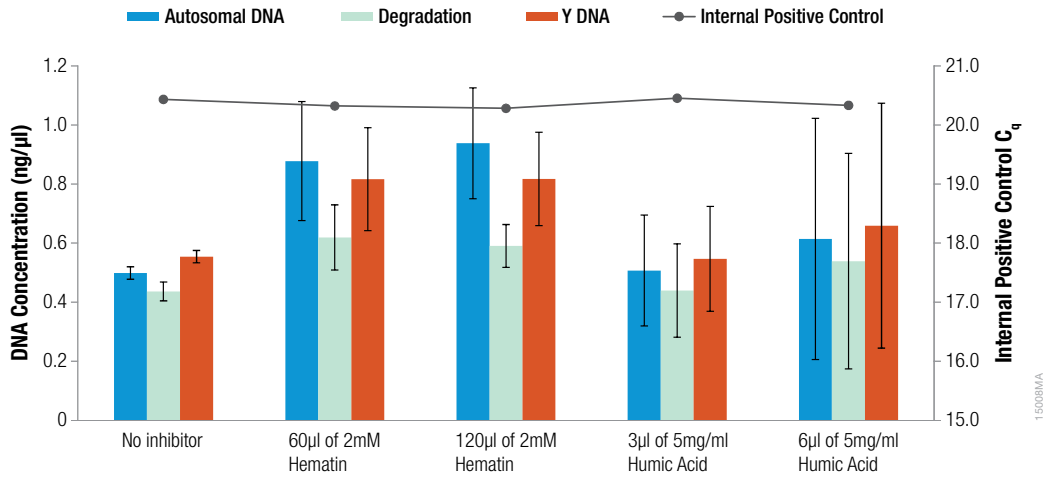

Figure 3. Quantification results for samples that contained hematin and humic acid inhibitors. The X axis represents the sample and the inhibitor present in the sample. The left Y axis represents the average DNA concentration in ng/µl (indicated by the bars). The right Y axis represents the average IPC C_q (indicated by the line). Error bars represent ±1 standard deviation. Samples were extracted using the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System.

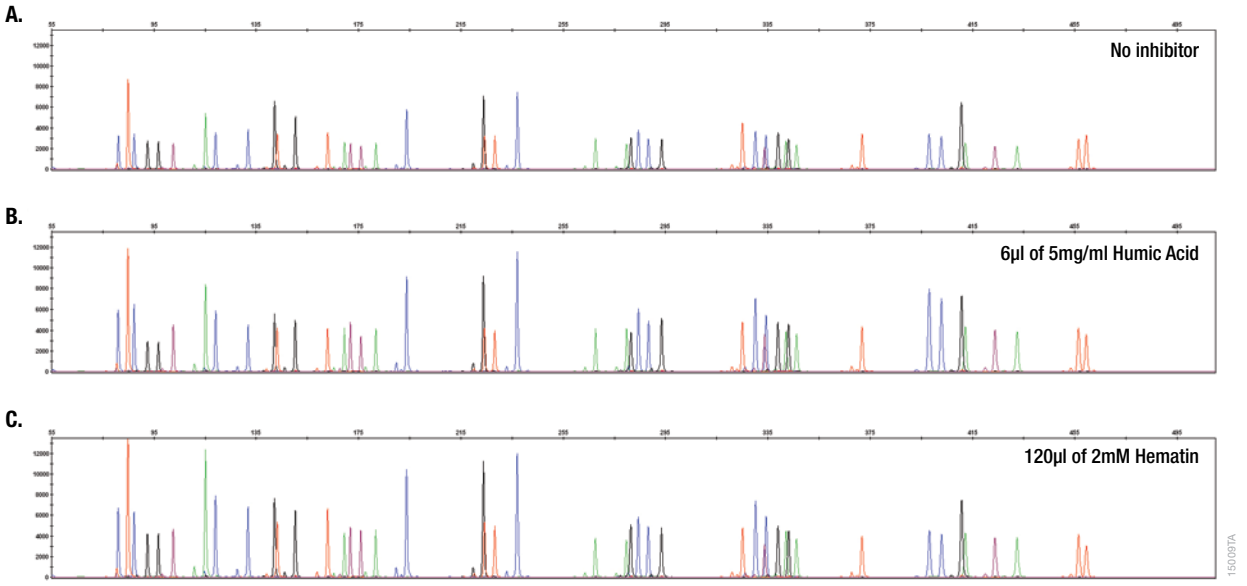

Figure 4. Representative DNA profiles of samples that contained humic acid and hematin inhibitors (scaled to 12,000RFU). Panel A. A DNA profile from a blood sample containing no added inhibitor. **Panel B.** A DNA profile from a blood sample containing 6µl of 5mg/ml humic acid. **Panel C.** A DNA profile from a blood sample containing 120µl of 2mM hematin. Samples were extracted using the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on a 3500xL series instrument using a 1.2kv, 24-second injection.

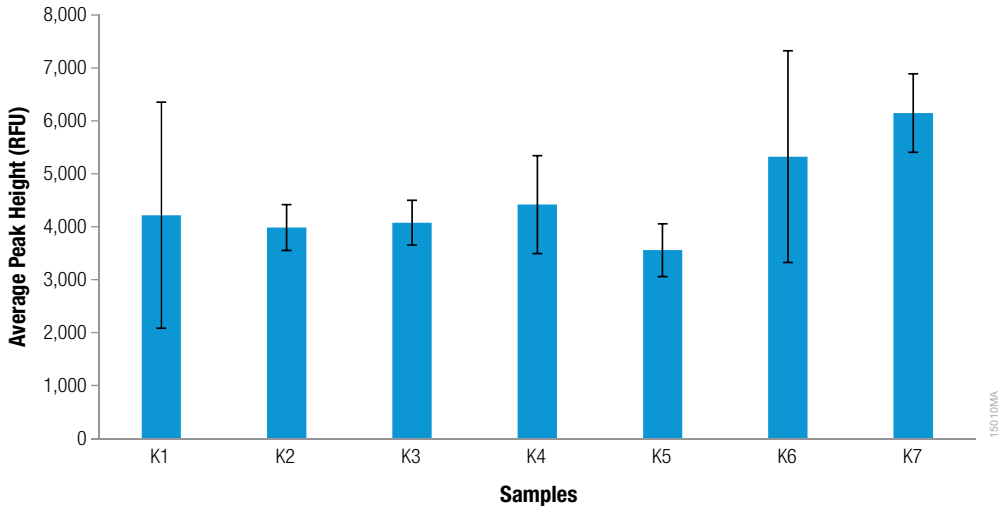

Figure 5. Average peak heights (from two replicate extractions) of known buccal samples. The X axis represents the samples. The Y axis represents the average peak heights in RFU, and error bars represent ±1 standard deviation. Samples were extracted using the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on a 3500xL series instrument using a 1.2kv, 24-second injection.

Known Samples

Full profiles were obtained for all known buccal samples, and average peak height ratio balance of the profiles ranged between 86% and 89% (data not shown). No IPC C_q flag was detected in any of the samples, indicating the extracted samples were free of inhibitors (data not shown). Amplification of the known samples with PowerPlex® Fusion 6C System resulted in expected average peak heights across all samples (Figure 5).

Casework Samples

The Maxwell® FSC Instrument was shown to successfully extract quantifiable DNA from a variety of sample types (Table 3). DNA yields were variable depending on sample type. However, the samples produced STR profiles with average peak heights consistent with DNA template amount. The IPC C_q flag was observed in one of two PowerQuant® replicates for a "blood in soil" sample. The STR profile generated from the sample with the IPC C_q flag was concordant with the reference profile and displayed no signs of low peak heights or imbalance. Analysis of the casework samples indicate the major contributor of each DNA profile is consistent with the expected male or female reference profile. A minor DNA profile of unknown origin was present in four samples (tennis shoes, t-shirt, ball cap and drinking straw), which can be expected with these sample types. For all differential extraction fractions, the major DNA profile was complete and matched the expected major contributor, while the minor alleles were consistent with the expected minor contributor. Representative electropherograms are shown for a mixed DNA sample (Figure 6), a low quantification sample (Figure 7) and a high quantification sample (Figure 8).

Table 3. Casework sample quantification and STR results. The asterisk (*) represents a flagged quality threshold in the PowerQuant® System. The STR data denote whether the PowerPlex® Fusion 6C System profile generated was of single-source or mixed composition and the percent profile obtained of the known source contributor.

Sample Description	[Auto] (ng/µl)	[Deg] (ng/µl)	[Y] (ng/µl)	[Auto]/[Y]	[Auto]/[D]	STR Data (% Expected Contributor Profile)
Steering Wheel Swab	0.0190	0.0029	0.0004	53.44*	6.48*	Single source ♀ (93%)
Steering Wheel Swab	0.0002	—	—	—	—	No profile
Gear Shift Swab	0.0031	0.0003	—	—	9.28*	Single source ♀ (41.5%)
Sweater Swab (inside neck and wrist)	0.0143	0.0053	—	—	2.71*	Single source ♀ (100%)
Glass Tea Bottle Swab (mouth area)	0.1227	0.0588	0.0001	1062.29*	2.09*	Single source ♀ (100%)
Inside Tennis Shoes Opening Swab	0.0425	0.0127	0.0008	54.96*	3.34*	Mixture. Major ♀ (100%)
T-Shirt Swab (inside neck area)	0.0224	0.0076	0.0010	21.40*	2.96*	Mixture. Major ♀ (100%)
Cell Phone Swab	0.0022	0.0003	—	—	6.51*	Single source ♀ (39%)
Phone Headset Swab	0.2525	0.0862	0.0015	168.34*	2.93*	Single source ♀ (100%)
Blood on Cotton	0.1486	0.1156	—	—	1.29	Single source ♀ (100%)
Blood on Cotton	0.2980	0.2941	0.2689	1.11	1.01	Single source ♂ (100%)
Blood on Denim	0.2845	0.2517	0.0004	702.85*	1.13	Single source ♀ (100%)
Blood on Denim	0.3681	0.3796	0.3533	1.04	0.97	Single source ♂ (100%)
Blood in Soil	0.9067	0.6724	—	—	1.35	Single source ♀ (100%)
Blood in Soil	3.5370	2.6829	—	—	1.32	Single source ♀ (100%)
Envelope Flap Cutting (~1 × 1cm)	0.0194	0.0126	—	—	1.54	Single source ♀ (100%)
Ball Cap Swab (inside rim area)	0.1222	0.0535	0.0545	2.24*	2.28*	Mixture. ♂ known contributor (100%)
Metal Drinking Straw Swab	0.2362	0.1028	0.0102	23.24*	2.3*	Mixture. Major ♀ (100%)
Golden Cigarette Filter Paper with 25µl Saliva	0.9639	0.3642	—	—	2.65*	Single source ♀ (100%)
Golden Cigarette Filter Paper with 25µl Saliva	3.0799	2.3422	—	—	1.31	Single source ♀ (100%)
White Cigarette Filter Paper with 25µl Saliva	0.7745	0.2419	—	—	3.20*	Single source ♀ (100%)
White Cigarette Filter Paper with 25µl Saliva	0.8259	0.5698	0.0003	3184.05*	1.45	Single source ♀ (100%)
♂ Buccal with Semen—Sperm Fraction	3.1188	3.0579	2.6011	1.20	1.02	Mixture. Major ♂ (100%)
♂ Buccal with Semen—Non-Sperm Fraction	22.3472	18.4697	20.3151	1.10	1.21	Mixture. Major ♂ (100%)
♀ Buccal with Semen—Sperm Fraction	13.0750	16.2538	13.5371	0.97	0.80	Single source ♂ (100%)
♀ Buccal with Semen—Non-Sperm Fraction	17.4023	15.2312	3.9420	4.41*	1.14	Mixture. Major ♀ (100%)
♀ Buccal with Semen—Sperm Fraction	0.9284	0.9454	0.7646	1.21	0.98	Mixture. Major ♂ (100%)
♀ Buccal with Semen—Non-Sperm Fraction	17.9656	12.4070	4.4332	4.05*	1.45	Mixture. Major ♀ (100%)

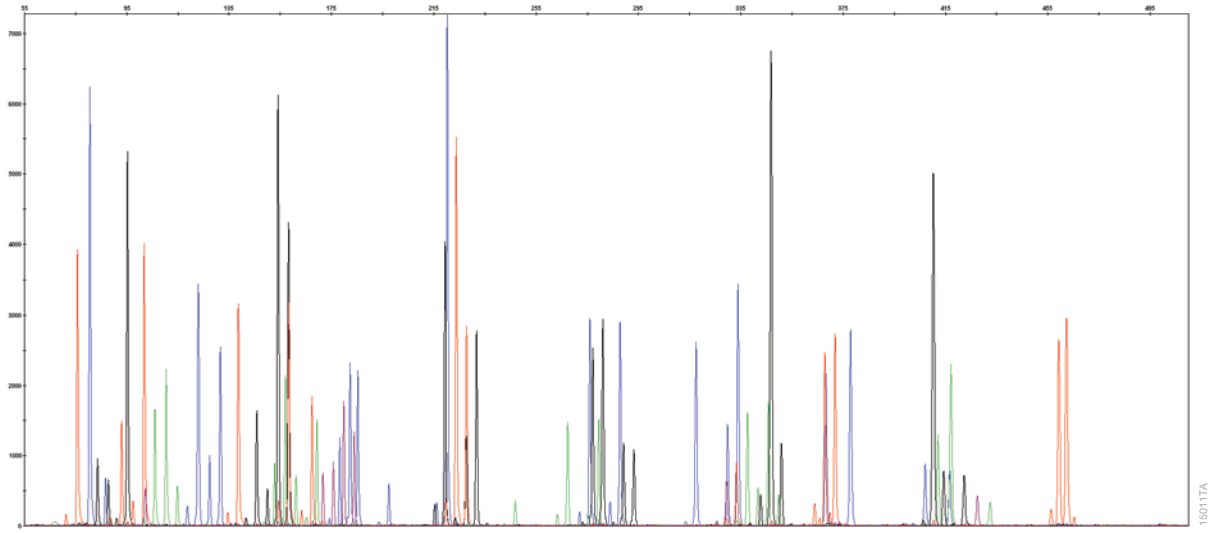

Figure 6. A mixed DNA profile from the non-sperm fraction of a female buccal sample mixed with semen (scaled to 7,000RFU). Sample was extracted using the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on a 3500 series instrument using a 1.2kv, 15-second injection.

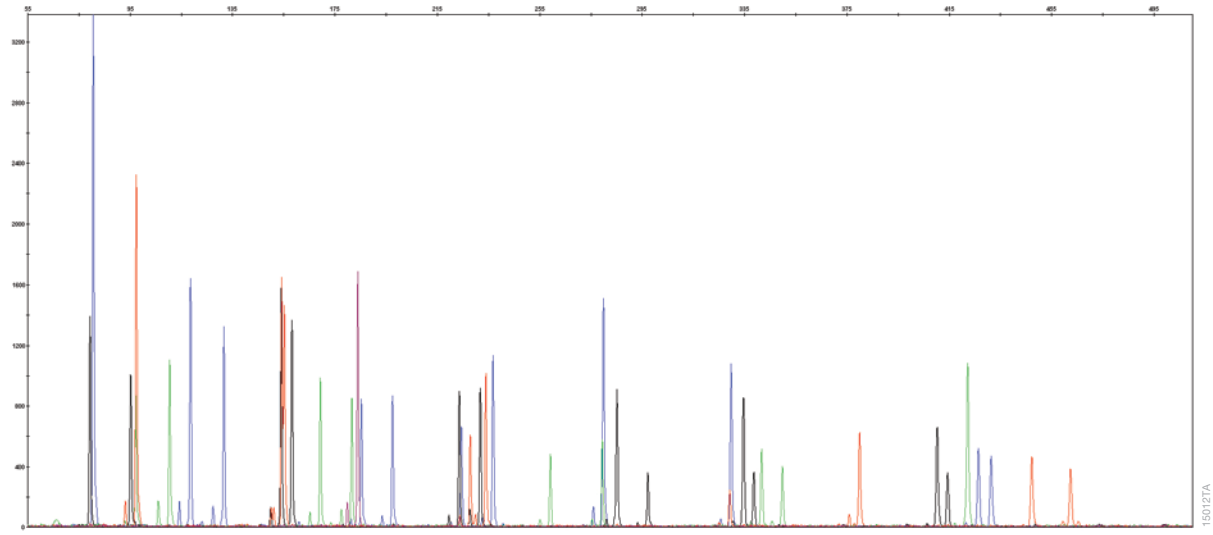

Figure 7. A low quantification (0.0143ng/µl) DNA sample from the swab of the interior of a sweater (scaled to 3,200RFU). Sample was extracted using the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on a 3500 series instrument using a 1.2kv, 15-second injection.

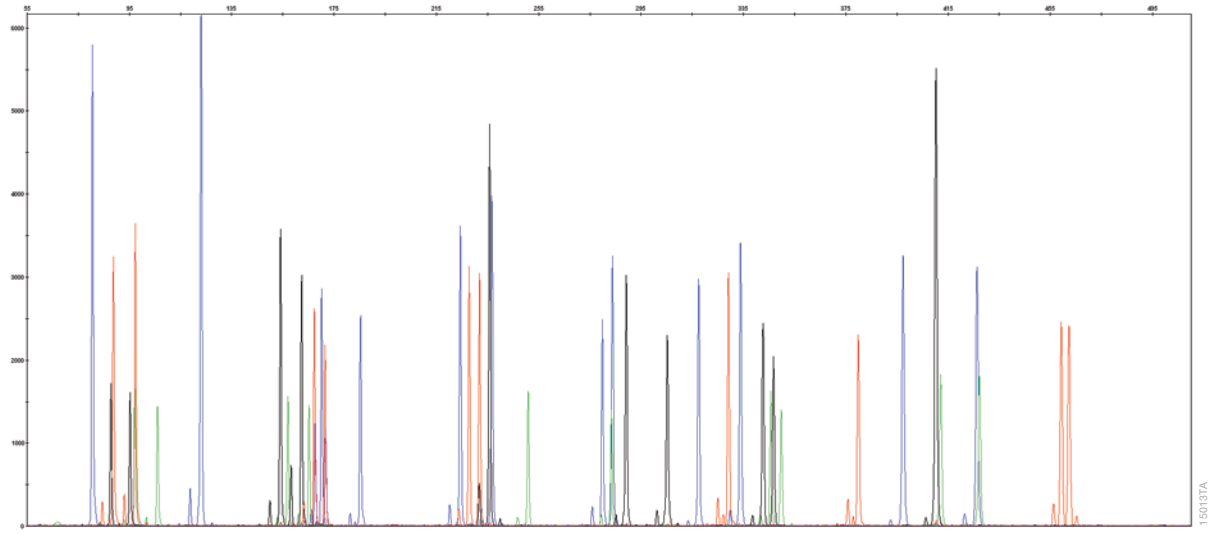

Figure 8. A high quantification (0.8259ng/µl) DNA sample from a white cigarette filter paper with 25µl of saliva (scaled to 6,000RFU). Sample was extracted using the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on a 3500 series instrument using a 1.2kv, 15-second injection.

Conclusion

Genomic DNA extraction using the Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® FSC Instrument was evaluated with a comprehensive set of experiments as recommended by current validation standards and guidelines. The results show that the system is capable of producing quality DNA extracts of reproducible yield and purity from a variety of casework samples and that the Maxwell® FSC Instrument is suitable for use within a forensic laboratory.

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